Trends in Analytical Chemistry 62 (2014) 20–27

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Analytical methods used in autism spectrum disorders

Joanna Kałużna-Czaplińska *, Jagoda Jóźwik, Ewa Żurawicz

Department of Chemistry, Institute of General and Ecological Chemistry, Lodz University of Technology, Lodz, Poland

ARTICLE INFO

Keywords:
Analytical method
Autism
Autism spectrum disorder
Autistic child
Biomarker
Chromatography
Etiology
Mass spectrometry
Metabolic profile
Trace element

ABSTRACT

Autism is a neurodevelopmental brain disorder characterized by deficiencies of language and social interaction skills, and repetitive behaviors, and is often accompanied by hyperactivity and limited attention. There are numerous theories about the specific causes of autism but they have not yet been proved. Many studies have indicated that autism has a broad multifactorial etiology, with predisposing factors including exposure to toxic chemicals, nutrient deficiencies, and genetic susceptibility. Analytical methods are important in the determination of metabolites in biological samples from autistic children for potential use as biomarkers of the disorder. Metabolic profiling helps to understand the etiology of autism and aids in the development of new treatments.

Contents

1. Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5), autism spectrum disorder (ASD) is characterized by deficits in social communication and social interaction and by the presence of restricted, repetitive patterns of behavior, interests, or activities. Because autism encompasses autistic disorder, Asperger's disorder, and pervasive developmental disorder, symptoms of these conditions comprise a single continuum of mild to severe impairments rather than being distinct. In the diagnosis of ASD, individual clinical characteristics are noted through the use of specifiers, which offer clinicians an opportunity to individualize the diagnosis. Thus, diagnosticians specify if ASD is associated with the presence of intellectual impairment, structural language impairment, known medical/genetic or environmental/acquired conditions, or another neurodevelopmental, mental, or behavioral disorder [1]. Medical testing of children with ASD has focused on four main areas – genetic testing, neuroimaging, electroencephalography (EEG), and metabolic screening.

Abbreviations: AAS, Atomic absorption spectroscopy; ASD, Autism spectrum disorder; CE-MS, Capillary electrophoresis–mass spectrometry; CE-TOF-MS, Capillary electrophoresis–time-of-flight mass spectrometry; DEHP, Di(2-ethylhexyl) phthalate; EEG, Electroencephalography; GC-MS, Gas chromatography–mass spectrometry; GC-MS-MS, Gas chromatography–tandem mass spectrometry; GI, Gastrointestinal; HPLC-NMR-MS, High-performance liquid chromatography–nuclear magnetic resonance spectroscopy–mass spectrometry; ICP-AES, Inductively-coupled plasma atomic emission spectroscopy; ICP-MS, Inductively-coupled plasma mass spectrometry; LC, Liquid chromatography; LC-MS, Liquid chromatography–mass spectrometry; LC-MS-MS, Liquid chromatography–tandem mass spectrometry; LLE, Liquid–liquid extraction; MS, Mass spectrometry; MT, Metallothionein; NMR, Nuclear magnetic resonance spectroscopy; SIM, Selective ion mode; SPE, Solid-phase extraction; SPME, Solid-phase microextraction; TMS, Trimethylsilyl; TMSO, Trimethylsilyl oxime; TOF-MS, Time-of-flight mass spectrometry; UHPLC, Ultra-high-performance liquid chromatography; UPLC-MS-MS, Ultra-performance liquid chromatography–tandem mass spectrometry; VPA, Valproic acid.

* Corresponding author. Tel.: +48 42 631 31 10; Fax: +48 42 631 30 91.
E-mail address: jkaluzna@p.lodz.pl (J. Kałużna-Czaplińska).

http://dx.doi.org/10.1016/j.trac.2014.06.014
0165-9936/

The use of analytical methods in the diagnosis of ASD emerges from the comorbidities and medical problems [including oxidative stress, inflammation, mitochondrial and gastrointestinal (GI) dysfunction] recorded in some people with ASD [2]. Recommendations for the identification of children with ASD with suggestive clinical findings include metabolic testing for lactate, pyruvate, and carnitine, acylcarnitine profile, liver and renal function, as well as for amino acids, urinary organic acids, lead, and serum ferritin [3,4]. It should be emphasized that powerful multivariate analytical methods are necessary for the development of complex "biomarker systems" aimed at assisting diagnosis, patient stratification, and predicting response to intervention in ASD [5–8]. In this regard, different analytical methods, notably gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) [6,7,9–11], play a key role. Other methods, such as inductively-coupled plasma MS (ICP-MS) and atomic absorption spectroscopy (AAS) are also used to determine elements in biological fluids of ASD children [12].

^{1}H-NMR is a rapid analytical method for establishing a metabolic profile, but only a relatively small number of metabolites can be identified by this method and it is less sensitive than MS [9]. MS is one of the best-known, highly sensitive and selective detection techniques, and gives information about the molecular structure of the compounds. The combination of GC or LC with MS can separate a mixture into its individual components and analyze each compound in the mixture both qualitatively and quantitatively [6,10]. In the case of GC, selective ionization mode (SIM) MS can be used to achieve high sensitivity. Low-molecular-weight metabolites are analyzed by GC-MS, but many compounds with polar groups need to be chemically derivatized before GC-MS analysis or LC-MS, and ultra-high-performance liquid chromatography (UHPLC) methods should be used. UHPLC shortens the time of analysis, often to 10 min or less [7]. Other MS techniques, such as time-of-flight (TOF-MS), may be used to aid the identification of metabolites. Quadrupole-TOF-MS allows the acquisition of full-scan product-ion spectra, giving accurate determinations of the mass of the product ions. A relatively new method, capillary electrophoresis with TOF-MS (CE-TOF-MS), can be used to measure metabolites of higher ionization and lower molecular weight, with easy preparation and high throughput [11].

ICP-MS is a flexible technique that offers many advantages over more traditional techniques for elemental analysis, such as AAS. The detection limits achievable for most elements are equivalent to or below those obtained by AAS. ICP-MS is a fast, multi-elemental technique and generally has the productivity of ICP atomic emission spectroscopy (ICP-AES) but with lower detection sensitivity [12].

This article reviews the current status of these analytical methods in establishing ASD biomarkers and in understanding the etiology of ASD.

2. Factors connected with autism spectrum disorder

2.1. Genetic factors

There is indisputable evidence that genes underlie the etiology of ASD. Research on identical twins suggests that, if one of the twins is autistic, the probability that the second will be autistic is 58% (males), 60% (females), whereas, in dizygotic twins, the probability drops to 21% (males) and 27% (females) [13].

Genetic screening tests are a powerful tool in the diagnosis of monogenic disorders with direct genotype–phenotype correlations. However, for ASD, widespread genetic testing would be not only costly and time consuming but also inadequate because of the complex etiology of ASD. But, genetic testing may be helpful in the diagnosis of genetic diseases with a high incidence of ASD comorbidity, such as fragile X syndrome, tuberous sclerosis, Angelman syndrome, neurofibromatosis type I, Rett syndrome, Down's syndrome, and phenylketonuria.

2.2. Environmental factors

Because identical twins are not fully concordant for ASD development, research has focused on other potentially contributing factors. These include mitochondrial disorders, environmental agents, infections during pregnancy, fetal testosterone levels, and parental age [14]. Attention has been drawn to the potential contributions of exposure to environmental toxins and mutagens during the early stages of development and in the immediate neonatal period [15], including the negative impact of maternal drug administration. Many drugs can cross the placenta and affect fetal development [16]. However, because of the diversity of drugs and medications, a direct relationship between fetal drug exposure and ASD development has been difficult to establish.

It was reported in 1994 that maternal use of thalidomide (sedative or anti-vomiting medication) during pregnancy was associated with not only limb deformities but also an ASD-like behavioral disorder in the offspring. Because most ASD victims of thalidomide also had deformities of the upper and lower limbs, it was argued that the time-window for abnormal fetal development leading to ASD is at a very early stage of pregnancy [17]. Another drug associated with the occurrence of ASD in offspring is Misoprostol (an agent used in the prevention of gastric ulcers and as an abortifacient) [18]. Maternal antiepileptic medication has also been implicated as a potential causal factor. Studies in animal models exposed to the anticonvulsant valproic acid (VPA) indicate that not only has VPA teratogenic effects but also rats exposed to VPA during gestation show deficiencies similar to those of ASD. Several primary ASD symptoms, including impairments in social interaction and higher sensitivity to stimuli, are present in rats treated prenatally with VPA [19]. Further research has suggested that the use of paracetamol (an analgesic and antipyretic) in children may be associated with increased risk of ASD [20].

Environmental exposure to di(2-ethylhexyl) phthalate (DEHP) has also been implicated as a risk factor for ASD development. DEHP is one of the most commonly used plasticizers in pharmaceutical and medical products, and elevated levels of DEHP metabolites have been reported in the urine of ASD children. Prenatal and postnatal exposure to phthalates is known to have a synergic detrimental effect on early brain development [21].

Furthermore, anomalies of Cu and Zn homeostasis have been reported in ASD, possibly indicative of deficient function of the metal-binding protein metallothionein (MT) [22,23]. MT plays diverse roles in not only heavy-metal detoxification but also neuronal development and immune response. It is possible that some of the common comorbidities of childhood ASD, including GI and respiratory disorders, could reflect defective MT function, and it has been suggested that MT protein dysfunction may be a primary cause of ASD [23].

There are clear indications that heavy metals may contribute to ASD development. For example, a case of overt lead poisoning in two autistic children has been described [24]. There is also direct evidence for abnormal processing of heavy metals in children with ASD. Analyses of heavy metals and trace elements in the body fluids and tissues of 25 children with ASD revealed significant differences compared to control children. Statistically significant differences were observed in hair samples in the levels of arsenic, cadmium, barium, cerium, lead, magnesium and zinc, and in urine samples for aluminum, barium, cerium, mercury, lead, copper and germanium. Parallel changes in barium and lead levels were seen in hair samples and urine. In contrast, significant differences in the average levels of arsenic, cadmium, cerium, magnesium and zinc in hair samples were not supported by the differences in the levels of these analytes in urine of autistics. Incompatibility in the levels